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Spontaneous Mutation and Parental Age in Humans

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SUMMARY

A statistical analysis of parental age and the incidence of new mutation has been performed. Some new data on Apert, Crouzon, and Pfeiffer syndromes is presented and combined with all available data from the literature on parental age and new mutation. Significant heterogeneity among syndromes for the rate of increase in incidence with parental age was found. A parsimonious conclusion is that mutations fall into two groups, one with a high rate of increase with age and the other with a low rate of increase with age. For the high-rate-of-increase group, a linear model relating incidence to age is rejected, while an exponential model is not. In addition, for this group, increased paternal age cannot account for the observed increase in maternal age—that is, increased maternal age also contributes to the incidence of new mutations. For the low-rate-of-increase group, increased paternal age alone can account for the observed increase in maternal ages; also, either a linear or exponential model is acceptable. In addition, there is no evidence for a mixture of parental age–independent cases with parental age–dependent cases for any of the syndromes examined. The curves reflecting incidence of new mutation and paternal age for two syndromes, Apert and neurofibromatosis, have an anomalous shape. In both cases the curve increases up to age 37 and drops at age 42 before increasing again at age 47. The usual explanation for the effect of parental age on new mutations is the mechanism of "copy-error" at mitotic division in male sper-

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matogenesis. These results cast doubt on this phenomenon as being the primary mechanism underlying the majority of spontaneous mutations for autosomal dominant disorders in man.

INTRODUCTION

Despite much theoretical speculation, the source of spontaneous mutation in humans remains unknown. An early clue was discovered by Weinberg (1912), who attributed increased parental ages in sporadic cases of achondroplasia to new dominant mutation. After the discovery of DNA and its method of replication, there appeared new theories as to the origin of the parental age effect for new mutations. Specifically, Penrose (1955) attributed the increased incidence of mutation with parental age to the continuous replication of stem cells in male spermatogenesis. He described a "copy-error" model, whereby stem cells would accumulate mutations with each replication. In an extensive review of the subject, Vogel (1956) and Vogel and Rathenberg (1975) have also conjectured that this type of model underlies the parental age effect for new mutations.

A critical issue is whether mutations occur primarily owing to replication-dependent or to replication-independent mechanisms. Uncorrected errors during replication of DNA provide a natural means for mutations to occur. If mutations are due to replication errors, then such errors are much more likely to occur in males than in females, since female germ cells have undergone far fewer cell divisions than male germ cells. Vogel and Rathenberg (1975) have used all available evidence to create a composite picture of oogenesis and spermatogenesis. In oogenesis, ~24 cell divisions have occurred to give rise to the full complement of a female's oocytes; in contrast, by puberty, 30 cell divisions have occurred among spermatozoa in males. Stem cells undergo mitotic divisions about every 16 days, resulting in 23 divisions/year. Hence, in a man aged 28, the spermatozoa would have undergone ~380 divisions and replications, which is approximately 15-fold greater than the corresponding number for oocytes. If stem-cell replications and divisions occur at a constant rate, then the total number of divisions underlying spermatozoa will be linear with paternal age, with an accumulation rate of ~23/year. Therefore, if most mutations are dependent on replication, then the vast majority should occur in sperm and not in ova, and the rate should increase with paternal age, independent of maternal age. Maternal age would appear to be increased only through correlation with paternal age. On the contrary, if most mutations are not replication dependent, then the mutation rates might be relatively similar in males and females, and any parental age effect could be attributable to both males and females.

Since the time of the Vogel and Rathenberg (1975) summary, additional data have appeared in the literature; we also have collected further data for a number of syndromes. Therefore, we believe the time is prudent for a critical

examination of the issue of spontaneous mutation and parental age. We start out with some theoretical developments relating to the "copy-error" model, expected increases in parental age, and examination of paternal versus maternal age effects. We then perform formal statistical analyses of our own data plus data collated from the literature regarding these issues and heterogeneity among and within syndromes.

The Copy-Error Model—Theoretical Aspects

In this section we attempt to characterize the relationship between paternal age t, the number of mitotic divisions experienced by a spermatid produced by a man of age t, and the probability that the spermatid carries a new mutation. We make the following definitions: $N(t)$ = the random number of mitotic divisions experienced by a spermatid in a man of age t; $f_t(x)$ = probability density function for $N(t)$; $F_t(x)$ = probability distribution function for $N(t)$; $\mu(t) = E[N(t)] = \int_0^\infty x f_t(x)\,dx$, where E is expectation; $\sigma^2(t) = \text{Var}[N(t)] = \int_0^\infty [x - \mu(t)]^2 f_t(x)\,dx$; $Q(n)$ = the cumulative probability of a new mutation existing in a spermatid after n mitotic divisions; and $\pi(t)$ = the probability that a spermatid produced by a man of age t carries a new mutation. Implicit in our formulation is the assumption that the probability of a new mutation depends on t only through n, the total number of mitotic divisions. We can then write the following equation:

$$\pi(t) = \int_0^\infty \text{Prob}[N(t) = x]Q(x)\,dx = \int_0^\infty f_t(x)Q(x)\,dx \ . \qquad (1)$$

1. Q(x) *is linear in* x.—Here we assume that the probability of a new mutation occurring at a mitotic division is independent of t. In this case, the same probability exists at each division, and so the cumulative probability of a mutation after x mitotic divisions is linear in x, namely,

$$Q(x) = b + a(x - t_0) \ ,$$

where t_0 may be some age (e.g., puberty) at which mitotic divisions begin. Then, from equation (1), we have

$$\pi(t) = \int_0^\infty f_t(x)Q(x)\,dx = \int_0^\infty f_t(x)[b + a(x - t_0)]\,dx = b + a[\mu(t) - t_0]. \qquad (2)$$

Thus, $\pi(t)$ is linearly related to the expectation $[\mu(t)]$ of $N(t)$ when $Q(x)$ is linear in x.

2. N(t) *as a renewal process.*—For more general functions $Q(x)$, we further characterize $N(t)$ as a renewal process. We make the following definitions: Y_i = the random time interval from the $i - 1$ to the ith mitotic division in a sperm stem cell; $E(Y_i) = \alpha_i = \alpha$ (assumed independent of i); and $\text{Var}(Y_i) = \beta_i^2 = \beta^2$ (assumed independent of i).

In addition, we assume that the Y_i, $i = 1, \ldots,$ are independent and identically distributed; that is, the time period between mitotic divisions does not depend on t. Then $N(t)$ constitutes a renewal process. By the Central Limit Theorem for Renewals (Feller 1968, eq. 12.10), we have

$$\frac{N(t) - t/\alpha}{\sqrt{t\beta^2/\alpha^3}} \to \Phi(x) \tag{3}$$

in distribution as $t \to \infty$; that is, the distribution function $F_t(x)$ converges asymptotically to a normal distribution with mean $\mu(t) = t/\alpha$ and variance $\sigma^2(t) = t\beta^2/\alpha^3$.

The normality conclusion does not require that the Y_i be identically distributed, since more liberal assumptions can be tolerated (e.g., Lindeberg's theorem; Feller 1968). Hence, it may be reasonable to assume normality of $N(t)$ for large values of t even though the Y_i have different means and variances (e.g., if α_i decreases with i).

3. *An exponential model for* Q(x).—Here we assume that Q has an exponential relationship with x, namely, $Q(x) = ce^{bx}$. Assuming normality for $F_t(x)$, we have, from equation (1):

$$\pi(t) = \int f_t(x)Q(x)\,dx$$

$$= \int \frac{1}{\sqrt{2\pi}\,\sigma(t)} e^{-1/2\{[x-\mu(t)]/\sigma(t)\}^2} ce^{bx}\,dx = ce^{b\mu(t)+1/2b^2\sigma^2(t)}.$$

According to formula (3), $\mu(t) = t/\alpha$ and $\sigma^2(t) = t\beta^2/\alpha^3$,

$$\pi(t) = ce^{bt/\alpha+1/2b^2t\beta^2/\alpha^3} = ce^{t(b/\alpha+1/2b^2\beta^2/\alpha^3)} = ce^{at},$$

where $a = b/\alpha + \frac{1}{2}b^2\beta^2/\alpha^3$. In other words, if $Q(x)$ is exponential in x, then the probability of a spermatid produced by a man of age t carrying a new mutation is also exponential in t. This relationship will also hold true when $b\sigma^2(t)$ is small relative to $\mu(t)$; for example, if there is little variation in the time between mitotic divisions of stem cells (β is small relative to α).

Other models relating probability of new mutation Q and number of cell divisions x are possible—for example, the power function $Q(x) = x^\alpha$. The case $\alpha = 2$ would have special significance, representing a "two-hit" model, in which each "hit" is linearly dependent on the number of cell divisions. In this case, however, π as a function of age, $\pi(t)$, would not necessarily be quadratic in age. According to equation (1), assuming a renewal process and normality for $F_t(x)$, we would have $\pi(t)$ proportional to $\mu^2(t) + \sigma^2(t)$. If, however, $\mu(t)$ and $\sigma^2(t)$ are both linear in t, or if $\sigma^2(t)$ is small relative to $\mu^2(t)$, and $\mu(t)$ is linear in t, then the relationship between π and t would also be quadratic.

Theoretically, the functions considered above for $\pi(t)$ increase without limit as $t \to \infty$. Because $\pi(t)$ is a probability, this is technically unrealistic, since the upper bound should be 1. Over the range of values considered here (say $t < 70$),

$\pi(t)$ should still be considerably below 1. The parameters of $\pi(t)$ are estimated from the observed number/expected number (O/E) ratio (see following section) and not from actual age-specific incidence data. Hence, absolute age-specific probabilities are not estimable; only relative probabilities are. Therefore, any value for rate of increase of probability with age is acceptable, since a sufficiently small (nonestimable) coefficient can be assumed to make the probability <1 at high age. In calculating the O/E ratio, this coefficient cancels from the numerator and denominator.

Derivation of the O/E Ratio

Studies of parental ages of new mutations compare the age distribution of parents of new mutants at the birth of the child with a suitable control-group parental age distribution (often the population census data). Usually, only a subset of all cases in a defined population are obtained, so age-specific incidence rates cannot be calculated. Instead, given the total number of cases, E in a given parental age interval is derived from the control demographics, and the O/E ratio for each interval is examined (Vogel and Rathenberg 1975). The O/E ratio is not necessarily directly comparable with the age-specific incidence curve. We make the following definitions: $\pi(i)$ = the probability of a new mutant occurring at parental age i; q_i = the proportion of children in the control sample with a parent of age i; $\mu_P = \Sigma i q_i$ = mean parental age in the control population; and $R(i) = O(i)/E(i)$ = the O cases with parental age i divided by the E cases with parental age i from the controls. Then

$$R(i) = \frac{q_i \pi(i) \Big/ \sum_j q_j \pi(j)}{q_i} = \frac{\pi(i)}{\sum_j q_j \pi(j)} .$$

Hence, $R(i)$ is a constant multiple of $\pi(i)$, the age-specific incidence function. Suppose $\pi(i)$ is linear in i. Let $\pi(i) = b + a(i - \mu_0)$. Then

$$R(i) = \frac{b + a(i - \mu_0)}{\sum_j q_j[b + a(j - \mu_0)]}$$

$$= \frac{b}{b + a(\mu_P - \mu_0)} + \frac{a}{b + a(\mu_P - \mu_0)} (i - \mu_0)$$

Hence, for $R(i)$, the slope γ with parental age i is

$$\gamma = \frac{a}{b + a(\mu_P - \mu_0)} .$$

Here μ_0 is defined as some age at which mitotic divisions begin (e.g., puberty, or $\mu_0 \simeq 13$). In general, $b \geq 0$, (representing a baseline probability of a new

mutation up to age μ_0), so that

$$\gamma = \frac{a}{b + a(\mu_P - \mu_0)} \leqslant \frac{1}{\mu_P - \mu_0} .$$

For most populations studied, the mean age of fathers, $\mu_F \geqslant 28$. Hence, if we take $\mu_0 = 13$, we get $\gamma \leqslant 1/15$. In other words, if the probability of a new mutation increases linearly with father's age, then the maximum slope of $R(i)$ is 1/15. As will be shown later, this value is considerably less than that observed for a number of syndromes.

If $\pi(i)$ is exponential, say $\pi(i) = be^{ai}$, then

$$R(i) = \frac{be^{ai}}{\sum_j q_j be^{aj}} = \frac{e^{ai}}{\sum_j q_j e^{aj}} = ce^{ai} .$$

Hence, the exponential rate of increase (a) for $R(i)$ is the same as that for $\pi(i)$.

Expected Mean Parental Age of New Mutants

First suppose that the relationship between parental age and the incidence of new mutations is linear—that is, that $\pi(i) = b + a(i - \mu_0)$. Then the mean age of parents of new mutations can be calculated as

$$\mu_P{}^A = \frac{\sum_i iq_i[b + a(i - \mu_0)]}{\sum_i q_i[b + a(i - \mu_0)]} = \frac{\mu_P[b + a(\mu_P - \mu_0)] + \sigma_P{}^2}{b + a(\mu_P - \mu_0)}$$

$$= \mu_P + \left(\frac{a}{b + a(\mu_P - \mu_0)}\right)\sigma_P{}^2 .$$

Here we again assume that μ_0 is the age of puberty. Assuming that $b \geqslant 0$, we have

$$\mu_P{}^A \leqslant \mu_P + \frac{1}{(\mu_P - \mu_0)}\sigma_P{}^2 . \qquad (4)$$

A special case of this relationship ($b = \mu_0 = 0$) was first observed by Penrose (see Smith 1972). According to formula (4), a linear model imposes a maximal increase in mean age of parents of new mutants, one that is independent of b, namely,

$$\frac{\sigma_P{}^2}{(\mu_P - \mu_0)} .$$

Assuming that $\mu_0 = 13$, the value of

$$\frac{\sigma_P^2}{(\mu_P - \mu_0)}$$

ranges from ~2.5 to 3.5 for most populations. As will be seen later, many syndromes show an increase in parental age far greater than this, putting the linear model into doubt. It is also straightforward to show that, for a linear model, the variance of parental age of new mutants is given by the formula

$$\tau_P^2 = \sigma_P^2 - \left[\frac{a\sigma_P^2}{b + a(\mu_P - \mu_0)}\right]^2 .$$

When $b = 0$ or is small compared to a, we have

$$\tau_P^2 = \sigma_P^2 - \left[\frac{\sigma_P^2}{(\mu_P - \mu_0)}\right]^2 . \tag{5}$$

For an exponential model for $\pi(i)$—for example, $\pi(i) = ce^{ai}$—the amount of increase in mean parental age depends on the value of a and is unlimited in its magnitude.

Paternal versus Maternal Age

The usual method for examining whether the parental age effect is attributable to fathers or mothers is partial correlations (Penrose 1933). Smith (1972) gave formulas to estimate simultaneously the effect of father's age, mother's age, and birth order on the incidence of new mutation. These formulas have been used extensively by investigators examining parental age effects. The Smith formula depends on the assumption of multivariate normality for the distribution of paternal age, maternal age, and birth order in the underlying population and an exponential increase in incidence with parental age. Let μ_F and σ_F^2 be the mean and variance, respectively, of paternal age and μ_M and σ_M^2 the mean and variance, respectively, for maternal age and ρ be the correlation between paternal and maternal age in the general population. Suppose that the effect of age is due entirely to fathers and that the incidence of new mutation is exponential with father's age—that is, that $\pi(i) = ce^{a_F i}$. Then, assuming bivariate normality for paternal and maternal age, Smith showed that the incidence of new mutations would also increase exponentially with maternal age with rate of increase $a_M = \beta_{FM} a_F$, where $\beta_{FM} = \rho\sigma_F/\sigma_M$ is the standard regression coefficient of paternal age on maternal age. It is important to note here that although the direct effect may be due to paternal age, the exponential rate of increase in the mother (a_M) could potentially be equal to or greater than that in the father (a_F), depending on the relative magnitudes of ρ, σ_F, and σ_M. Smith also showed that the expected increase in maternal age could be written in

terms of the increase in paternal age and the regression of paternal on maternal age, as

$$\mu_M{}^A - \mu_M = \beta_{MF}(\mu_F{}^A - \mu_F) \ .$$

The above arguments continue to apply if the direct effect is on mothers rather than on fathers, with subscripts F and M reversed.

Unfortunately, the bivariate-normality assumption for paternal and maternal ages is unwarranted. In most populations, the maternal age distribution may approximate normality; however, the paternal age distribution is positively skewed and in fact shows closer correspondence to a square-root normal distribution. This may be due, in large part, to the biological limitations placed on maternal age, limitations that do not exist for paternal age. In addition, the correlation between paternal and maternal age in general decreases with increasing paternal age, so that, for example, mean age of wives of fathers of age 45 is not much different from that of wives of fathers of age 55. Also, the conditional variance of maternal age given paternal age is not constant but increases with paternal age.

What is the impact of lack of bivariate normality on conclusions about the relative contribution of paternal and maternal age to the incidence of new mutations? The bivariate-normality assumption will lead to an overestimate of the expected maternal age for a given paternal age when paternal age is high; hence, the effect of maternal age will be underestimated. The extent of underestimation will depend on the magnitude of the parental age effect. For mutations with a modest parental age effect (exponential rate of increase a is small), the discrepancy is minor; when a large parental age effect (large a value) is present, the discrepancy will be large; for example, we have examined the discrepancy between expected maternal age assuming bivariate normality and using the direct bivariate distribution of parental age for the United States censuses from 1960 to 1980. Table 1 shows that when $a = .05$ the discrepancy (maternal age assuming bivariate normality minus true value) is on the order of 0.1 years; when $a = .10$, the discrepancy is $\sim$0.55 years; and when $a = .15$, the discrepancy is $\sim$1.52 years. Therefore, especially when a is large, the bivariate-normality assumption is inadequate for assessing the role of paternal and maternal ages, and only the direct bivariate parental age distribution should be used.

DATA

New Cases

We include in our analysis 68 new cases of fresh mutations—26 with Apert syndrome, 22 with Crouzon syndrome, and 20 with Pfeiffer syndrome. All cases were evaluated at the New York University Institute for Reconstructive Plastic Surgery (by J.G.M.) and at the Genetics Clinic (by E.W.R. and M.M.W.), and positive family history was definitively ruled out. Any question-

TABLE 1

DIFFERENCE BETWEEN MATERNAL AGES PREDICTED FROM (1) BIVARIATE NORMAL ASSUMPTION AND (2) ACTUAL BIVARIATE DISTRIBUTION FOR PATERNAL EFFECT MODEL: 1950–80 U.S. CENSUSES

	a		
CENSUS YEAR	0.05	0.10	0.15
1950	0.10	0.51	1.38
1960	0.10	0.55	1.54
1970	0.08	0.50	1.49
1980	0.12	0.62	1.79

able cases were not included. For all cases, both paternal and maternal ages at birth of the child were known.

To obtain an appropriate control population for our three groups of cases, we determined the year of birth of each of our cases and used the bivariate parental age distribution for that year (in 5-year intervals) from the U.S. census. A relatively equal number of patients were born in the years from 1960 to the present and relatively fewer between 1950 and 1960. The control bivariate parental age distribution was obtained by weighting the appropriate census year by the proportion of cases born in that year (in 5-year intervals). This approach is similar to that used by Riccardi et al. (1984).

Literature Cases

We have also included in our analysis all cases reported in the literature of new mutations for autosomal dominant disorders. We have included only those studies that describe an appropriate control population or how to obtain such a population for their cases. Unfortunately, this has led to the exclusion of a few of the classical studies; however, we decided that an adequate control group was a prerequisite for these analyses. In 1975, Jones et al. (1975) summarized much of the literature on parental age and new mutations; however, they gave only mean and SDs (not actual ages) for the various syndromes. In doing our analyses we have eliminated overlap between original reports and the summary of Jones et al. (1975). For control data for retinoblastoma, Pellie et al. (1973) used the French national census, which gives paternal and maternal age distributions separately. We have used the bivariate Australian census data for 1953, because these data had mean maternal and paternal ages identical to those in the French census data.

We have only used studies that included >10 cases. The list of studies and the corresponding control populations used are given in table 2. It is impossible to assume that the control samples are precisely matched to the cases in terms of demographics. However, we believe that no significant systematic distortion due to use of inappropriate control samples has occurred in our results.

TABLE 2

STUDIES USED IN THE ANALYSIS OF PARENTAL AGE AND NEW MUTATION, BY SYNDROME

Syndrome and Source	n_f	n_m	Controls
AP:			
Murdoch et al. 1970	106	107	U.S. census, weighted by year
Stevenson 1957	46	46	Hospital-matched controls
AD:			
Jones et al. 1975	11	11	U.S. census, 1966
Apert:			
Present study	26	26	U.S. census, weighted by year
Cohen 1975	48	48	U.S. census, weighted by year
Blank 1960	37	37	Australian census, 1953
BR:			
Pellie et al. 1973	155	155	Australian census, 1953
BCN:			
Jones et al. 1975	12	12	U.S. census, 1955
CCD:			
Jones et al. 1975	32	32	U.S. census, 1966
Crouzon:			
Present study	22	22	U.S. census, weighted by year
Jones et al. 1975	38	38	U.S. census, 1966
FOP:			
Rogers and Chase 1979	38	40	U.S. census, 1960
Connor and Evans 1979	33	33	Australian census, 1953
Tunte et al. 1967	39	38	French census, weighted by year
Marfan:			
Murdoch et al. 1972	23	23	U.S. census, 1955
ME:			
Jones et al. 1975	14	14	U.S. census, 1966
NF:			
Riccardi et al. 1984	187	171	U.S. census, weighted by year
Sergeyev 1975	56	56	Moscow census, weighted by year
ODD:			
Jones et al. 1975	11	11	U.S. census, 1966
Pfeiffer:			
Present study	20	20	U.S. census, weighted by year
Progeria:			
Jones et al. 1975	18	18	U.S. census, 1966
Sotos:			
Jones et al. 1975	50	50	U.S. census, 1966
T-C:			
Jones et al. 1975	98	98	U.S. census, 1966
Waardenburg:			
Jones et al. 1975	22	22	U.S. census, 1955

NOTE.—AP = achondroplasia; AD = acrodysostosis; BR = bilateral retinoblastoma; BCN = basal cell nevus; CCD = cleidocranial dysostosis; FOP = fibrodysplasia ossificans progressiva (or myositis ossificans); ME = multiple exostoses; NF = neurofibromatosis; ODD = oculo-dento-digital; and T-C = Treacher-Collins.

METHODS

Linear Model

We first compared the mean paternal age predicted by a linear model with that observed in each of the data sets. We did the same for maternal age, assuming that the effect was related to maternal age directly rather than related via correlation with paternal age.

To obtain the predicted paternal ages, we applied formula (4) to the control population, assuming that $a = 0$ and $\mu_0 = 13$ (the age of puberty). This approach is conservative: a value of $a > 0$ or a value of $\mu_0 < 13$ (e.g., $\mu_0 = 0$) would give smaller predicted mean parental ages. Hence, any values significant under these assumptions would also be significant under any alternative conditions. Significance was assessed by means of a Z-test, assuming that

$$Z = \frac{\mu_P{}^0 - \mu_P{}^A}{\tau_P/\sqrt{n}}$$

has an approximate normal distribution with mean 0 and variance 1, where $\mu_P{}^0$ is the observed parental age, $\mu_P{}^A$ is the mean age predicted by the linear model, τ_P is the SD of parental age predicted from the linear model applied to the control population (see eq. [5]), and n is sample size.

O/E Ratios

The control distribution of parental ages was used to calculate the E parents in a given age interval. For those studies that provided the actual parental ages, we calculated and plotted the O/E ratio of parents within each 5-year age interval. For multiple data sets on the same disorder, we combined E and O, and calculated a total O/E ratio. This analysis was performed for fathers and mothers separately.

Exponential Model

We examined an exponential model for each sex separately. We fit an exponential curve and estimated a by using maximum likelihood as follows:

The probability that a father of a new mutant will be age i is

$$q_i e^{ai} \Big/ \sum_j q_j e^{aj} \ .$$

Suppose that r_i fathers are observed to be age i. Then the likelihood can be written, aside from a constant, as

$$L = \prod_i \left(\frac{q_i e^{ai}}{\sum_j q_j e^{aj}} \right)^{r_i} \ .$$

Thus,

$$\ln L = aT - R\ln\left(\sum_j q_j e^{aj}\right) ,$$

where

$$R = \sum_j r_j \text{ and } T = \sum_j j r_j .$$

$$\frac{\partial \ln L}{\partial a} = T - R\frac{\sum_j j q_j e^{aj}}{\sum_j q_j e^{aj}} .$$

Hence, the maximum-likelihood estimate $\hat{a}$ is obtained by solving

$$\frac{\sum_j j q_j e^{aj}}{\sum_j q_j e^{aj}} = T/R \ (= \mu^{\circ}, \text{ the observed parental mean age}),$$

which was done by iteration.

The variance of $\hat{a}$ was estimated by calculating

$$-\left(\frac{\partial \ln L}{\partial a}\right)^{-2} .$$

Analysis of mothers was performed in a similar fashion.

Note that the maximum-likelihood estimator $\hat{a}$ depends only on the observed mean and that the information depends only on the mean and sample size R. For those studies that provided actual parental age distributions, we classified ages into 5-year intervals and assessed goodness-of-fit of the exponential model by a χ^2-test.

Heterogeneity among Syndromes and Studies

Heterogeneity of the estimated a value among different studies of the same syndrome and among different syndromes was assessed as follows. For each study, we assumed that the estimate $\hat{a}$ was approximately normally distributed with its calculated SE. Comparing n studies, we first calculated a weighted average a value as

$$\bar{a} = \left(\sum_{i=1}^{n} a_i/s_i^2\right) \Big/ \left(\sum_{i=1}^{n} 1/s_i^2\right) ,$$

where i ranges over the n studies, and a_i and s_i are the estimate of a and its SE in the ith study, respectively. Heterogeneity among the n studies was then assessed by calculating

$$\chi^2 = \sum_{i=1}^{n} \left(\frac{a_i - \bar{a}}{s_i}\right)^2 ,$$

which has a χ^2 distribution with $n - 1$ df under the null hypothesis of no heterogeneity.

If heterogeneity was significant among different syndromes, then we wished to determine the minimum number of intrinsic a values that were necessary to account for the variation. Therefore, we assumed a heterogeneity model whereby there are m intrinsic a values, for which the jth one has value A_j and occurs among a proportion p_j of syndromes; note that

$$\sum_{j=1}^{m} p_j = 1 .$$

The likelihood of the n observed a values $(a_1, \ldots, a_n)$ is then given by

$$L = \prod_{i=1}^{n} \left[\sum_{j=1}^{m} p_j \frac{1}{\sqrt{2\pi}\sigma_i} e^{-1/2(a_i - A_j/\sigma_i)^2}\right] .$$

The likelihood and estimates of p_j and A_j were calculated for $m = 1, 2, \ldots$. At each stage a likelihood-ratio test was performed to determine whether the inclusion of an additional A value significantly improved the likelihood. In each case, since two additional parameters (p and A) were estimated, minus twice the log-likelihood difference has a χ^2 distribution with 2 df. In this way, the minimum number of A values necessary to explain the observed variation among the estimated a values was assessed.

Mutation-Heterogeneity Model

We also examined a mutation-heterogeneity model, as follows. Suppose that some mutations are exponentially dependent on paternal age, while the remainder are independent of paternal age. Then the incidence curve of new mutations with paternal age has the form $\pi(i) = d + ce^{ai}$, where d is the age-independent incidence of new mutations. In fact, d and c are not separately estimable since we do not have absolute incidence rates. What we can estimate is a and the parameter c/d (equivalent to fixing $d = 1$). This type of model would be appropriate if only a subset of mutations were exponentially related to paternal age (e.g., mothers contribute some age-independent new mutations). According to this model, the probability that a father of a new mutant is of age i is

$$\frac{q_i(1 + ce^{ai})}{\sum_j q_j(1 + ce^{aj})} .$$

The likelihood of the observed paternal ages can be written analogously to the simple exponential model described above, and maximum-likelihood estimates of a and c can be obtained.

Paternal versus Maternal Age

To assess the contribution of paternal and maternal age to the incidence of new mutations, we used a general mixed-effect exponential model. In this model we assumed that mutations can occur in either parent and that the prior probability of a new mutant occurring in the father is some parameter p, while the remainder (1 − p) occur in the mother. We assumed the same exponential relationship (i.e., $\pi(i) = ce^{ai}$) between incidence of new mutation π and age i for both sexes. Therefore, according to this model, the observed paternal age distribution is determined partly by direct exponential effect on paternal age and partly through correlation with increased maternal age. A comparable statement applies to the maternal age distribution.

Three important submodels correspond to p = 1 (a paternal effect model with mutation occurring only in males), p = 0 (a maternal effect model with mutation occurring only in females), and p = .5 (mutation occuring with equal prior probability in both sexes). Of course, other values of p are possible.

According to the mixed-effect model, the mean paternal and maternal ages can be calculated as follows. Define q_{ij} as the proportion of control offspring with a father of age i and a mother of age j. Then, according to the mixed-effect model, the probability of observing that a father of a new mutant has age i is

$$\mathrm{p}\left(\frac{\sum_j q_{ij}e^{ai}}{\sum_{i,j} q_{ij}e^{ai}}\right) + (1 - \mathrm{p})\left(\frac{\sum_j q_{ij}e^{aj}}{\sum_{i,j} q_{ij}e^{aj}}\right).$$

Therefore, the expected mean paternal age is

$$\mu_F{}^E = \mathrm{p}\left(\frac{\sum_{i,j} iq_{ij}e^{ai}}{\sum_{i,j} q_{ij}e^{ai}}\right) + (1 - \mathrm{p})\left(\frac{\sum_{i,j} iq_{ij}e^{aj}}{\sum_{i,j} q_{ij}e^{aj}}\right).$$

An analogous formula can be derived for $\mu_M{}^E$.

To test the three models described above (p = 1.0, .5, and 0), we calculated the value of a that coincided with the observed mean paternal age for that syndrome and then determined the corresponding expected mean age for mothers. The observed versus expected mean maternal ages were compared by means of a Z-test as described above for the linear model. In this case, however, the expected conditional SD (mother given father) was used. The conditional SD is not constant but, in general, increases with paternal age. Therefore, we calculated the expected conditional SD directly from the control

population, assuming an exponential model for the observed mean paternal age. We also estimated the values of a and p that yielded simultaneously the observed mean paternal and maternal ages.

When necessary, all likelihood maximizations were obtained with the computer program MAXLIK (Kaplan and Elston 1972).

RESULTS

Linear Model

The observed parental ages and SDs from 24 studies are given in table 3. Parental ages predicted from the linear model assuming $\mu_0 = 13$ and $a = 0$ are also given in table 3, along with the corresponding Z-statistic for each. The predicted maternal ages are based on a direct linear model applied to mothers (i.e., they are not determined through correlation with father's ages). Predicted maternal ages would be correspondingly lower assuming a linear model applied to fathers. As can be seen in the table, for 19 of the 24 studies, the linear model underpredicts mean paternal age. In 10 of the samples, the deviation is significant ($P < .05$). Combining evidence across studies, we can also reject a linear model for fibrodysplasia ossificans progressiva ($Z = 2.03$, $P < .05$). Among mothers, there is also a tendency for underprediction by a linear model; in 14 of the 23 studies, mean maternal age is underpredicted. In four of these studies the difference is significant; however, in three studies maternal age is significantly overpredicted. These results are conservative; for example, if we assume that $\mu_0 = 0$ rather than that $\mu_0 = 13$, then mean paternal age is underpredicted in all studies and the difference is significant in 16 of 24 of them. We conclude that a linear model relating incidence to paternal age is inadequate for explaining the parental age effect for the majority of syndromes.

O/E Ratios

Rejection of the linear model is confirmed in the examination of the O/E ratios for those studies that included parental age distributions. The E parents in 5-year age intervals calculated from the control sample, the O parents, and thus the O/E ratio are given in table 4. Fathers and mothers are presented separately. Data were available for seven syndromes: Apert, fibrodysplasia ossificans progressiva, neurofibromatosis, achondroplasia, Marfan, Pfeiffer, and Crouzon. The O/E ratios are also plotted in figures 1 (fathers) and 2 (mothers). In the Introduction we showed that under a linear model the maximum slope that the curve can achieve is $1/(\mu_P - \mu_0) \leq 1/15$. Hence, if we assume a value of O/E near 0 at age 17, then the maximum possible value at age 47 would be 2.0 and that at age 52 would be 2.33. For all of the syndromes plotted in figure 1, the observed O/E values at ages 47 and 52 are greater than those consistent with a linear model (only neurofibromatosis is possibly compatible with a linear model). Again, these conclusions are conservative; if we assume that $\mu_0 < 13$ (e.g., $\mu_0 = 0$), then discrepancies with a linear model are greater. For mothers, a linear function also appears incompatible with the observed curves, with the possible exceptions of neurofibromatosis and Crouzon syndrome.

TABLE 3

OBSERVED AND EXPECTED PARENTAL AGES FOR LINEAR MODEL STARTING AT PUBERTY

SYNDROME: SOURCE	OBSERVED		LINEAR-MODEL PREDICTION			
	$\mu_F(\sigma_F)$	$\mu_M(\sigma_M)$	μ_F^A	Z_F	μ_M^A	Z_M
Apert: present study	35.9(8.3)	31.0(6.1)	31.5	3.41**	28.2	2.54***
Pfeiffer: present study	35.9(9.7)	29.6(5.6)	31.5	2.99**	28.2	1.11
BCN: Jones et al. 1975	36.9(5.9)	31.7(6.4)	32.9	2.01*	29.2	1.46
Apert: Blank 1960	36.9(7.7)	31.8(4.9)	33.6	3.04**	30.0	1.92*
AP: Stevenson 1957	38.9(10.4)	32.4(6.5)	33.1	5.74***	30.4	2.35**
Marfan: Murdoch et al. 1972	36.6(9.1)	29.3(5.4)	32.9	2.58**	29.2	0.08
Apert: Cohen 1975	35.7(8.3)	29.5(6.1)	32.7	3.03**	29.1	0.46
Progeria: Jones et al. 1975	34.1(8.9)	29.7(6.4)	31.9	1.34	28.4	0.93
Crouzon: Jones et al. 1975	33.9(8.3)	28.6(5.9)	31.9	1.77*	28.4	0.21
FOP: Tunte et al. 1967	36.3(7.7)	30.8(7.4)	34.3	1.85*	30.9	−0.10
AP: Murdoch et al. 1970	36.2(7.6)	30.6(6.1)	33.9	3.22***	29.6	1.66*
Waardenburg: Jones et al. 1975	34.8(9.0)	30.5(7.0)	32.9	1.30	29.2	1.02
FOP: Connor and Evans 1979	35.0(6.8)	30.4(4.6)	33.6	1.22	30.0	0.40
CCD: Jones et al. 1975	33.1(7.8)	29.8(6.8)	31.9	0.98	28.4	1.33
AD: Jones et al. 1975	33.0(6.1)	28.1(5.7)	31.9	0.52	28.4	−0.17
NF: Riccardi et al. 1984	32.8(8.3)	27.4(6.0)	32.1	1.42	28.6	−2.71**
FOP: Rogers and Chase 1979	33.0(7.6)	28.5(6.0)	32.5	0.45	29.0	−0.53
ODD: Jones et al. 1975	32.3(5.6)	28.1(4.4)	31.9	0.19	28.4	−0.17
Crouzon: present study	31.8(9.0)	27.1(4.6)	31.5	0.21	28.2	−0.91
Sotos: Jones et al. 1975	31.7(7.9)	28.8(6.7)	31.9	−0.20	28.4	0.47
T-C: Jones et al. 1975	31.2(7.0)	27.0(6.0)	31.9	−0.99	28.4	−2.31*
NF: Sergeyev 1975	32.9(6.4)	29.9(6.1)	33.5	−0.67	31.0	−1.35
ME: Jones et al. 1975	30.6(5.0)	26.1(5.0)	31.9	−0.70	28.4	−1.44
BR: Pellie et al. 1973	32.3(7.1)	28.2(5.4)	33.6	−2.44**	30.0	−3.93***

NOTE.—Syndrome acronyms are as in table 2.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

TABLE 4

EXPECTED (E), OBSERVED (O), AND EXPONENTIAL MODEL PREDICTION (P) FOR PARENTAL AGE DISTRIBUTIONS, BY SYNDROME

SYNDROME, SOURCE AND DISTRIBUTION	PATERNAL AGE								MATERNAL AGE						
	<24	25–29	30–34	35–39	40–44	45–49	>50	χ^2_5	<19	20–24	25–29	30–34	35–39	>40	χ^2_4
Apert:															
Present study:															
E	7.99	8.22	5.38	2.66	1.13	0.41	0.22		3.40	9.20	7.50	3.90	1.60	0.41	
O	2	5	3	10	2	1	3		0	3	8	7	7	1	
O/E	0.25	0.61	0.56	3.76	1.77	2.44	13.64		0.0	0.33	1.07	1.79	4.38	2.44	
P	2.53	4.84	5.52	4.76	3.52	2.24	2.57	8.45	0.73	3.95	6.40	6.61	5.42	2.88	3.07
Blank 1960:															
E	5.61	11.94	9.50	5.59	2.90	1.04	0.41		1.76	10.51	12.06	7.72	3.83	1.13	
O	1	4	9	14	5	2	2		0	3	9	14	10	1	
O/E	0.19	0.34	0.95	2.50	1.72	1.92	4.88		0.0	0.29	0.75	1.81	2.61	0.88	
P	2.02	6.79	8.41	7.70	6.22	3.48	2.39	9.25	0.48	4.78	9.22	9.95	8.32	4.25	5.62
Cohen 1975:															
E	12.07	14.34	10.62	6.33	3.01	1.11	0.52		5.36	15.83	13.28	8.15	4.18	1.20	
O	3	10	12	10	3	8	2		1	8	17	10	9	3	
O/E	0.25	0.70	1.13	1.58	1.00	7.21	3.85		0.19	0.51	1.28	1.23	2.15	2.50	
P	4.63	8.90	10.27	9.54	7.08	4.05	3.52	7.88	2.15	9.63	12.21	11.32	8.78	3.91	3.14
Total:															
E	25.67	34.50	25.50	14.57	7.04	2.56	1.15		10.52	35.54	32.83	19.76	9.62	2.73	
O	6	19	24	34	10	11	7		1	14	34	31	26	5	
O/E	0.26	1.55	0.94	2.33	1.42	4.30	5.20		0.10	0.39	1.04	1.57	2.70	1.83	
P	9.24	20.24	24.03	22.06	17.13	9.99	8.52	13.04**	3.27	18.60	28.01	27.93	22.52	10.97	7.99
FOP:															
Connor and Evans 1979:															
E	5.01	10.65	8.47	4.99	2.59	0.93	0.36		1.57	9.37	10.75	6.89	3.42	1.00	
O	0	6	12	8	5	0	2		0	1	15	9	7	1	
O/E	0	0.56	1.42	1.60	1.93	0	5.52		0.0	0.11	1.40	1.31	2.05	1.00	
P	2.23	6.92	7.92	6.71	5.01	2.60	1.61	7.40	0.57	5.13	8.95	8.72	6.58	3.05	9.40
Rogers and Chase 1979:															
E	10.42	11.16	8.19	4.83	2.19	0.81	0.40		5.05	13.48	10.49	6.62	3.45	0.91	
O	6	8	10	6	4	4	0		4	9	12	6	6	3	
O/E	0.58	0.72	1.22	1.24	1.83	4.84	0.0		0.79	0.67	1.14	0.91	1.74	3.30	
P	6.00	8.84	8.67	6.82	4.13	2.03	1.50	3.80	2.98	10.31	10.41	8.52	5.77	2.02	1.99

Tunte et al. 1967:															
E	3.11	6.21	6.46	4.19	2.00	0.74	0.29		0.83	6.11	7.08	4.93	2.95	1.11	
O	1	1	8	4	4	4	1		1	3	4	6	7	2	
O/E	0.32	0.16	1.24	0.95	2.00	5.41	3.45		1.20	0.49	0.56	1.22	2.37	1.80	
P	0.90	3.03	5.13	5.39	4.19	2.50	1.85	4.63	0.30	3.31	5.64	5.77	5.07	2.90	3.16
Tunte et al. 1967:															
E	2.17	4.32	4.50	2.91	1.39	0.51	0.20		0.54	3.99	4.62	3.21	1.92	0.72	
O	2	2	4	2	4	1	1		0	6	3	1	2	3	
O/E	0.92	0.46	0.89	0.69	2.88	1.96	5.00		0.0	1.50	0.65	0.31	1.04	4.17	
P	0.96	2.78	4.06	3.68	2.47	1.27	0.77	3.19	0.33	2.99	4.23	3.60	2.63	1.23	8.29
Total:															
E	20.71	32.34	27.63	16.91	8.17	2.99	1.25		7.98	32.95	32.94	21.64	11.74	3.75	
O	9	17	34	20	17	9	4		5	19	34	22	22	9	
O/E	0.43	0.53	1.23	1.18	2.08	3.01	3.21		0.63	0.58	1.03	1.02	1.87	2.40	
P	9.48	21.67	26.67	22.98	15.81	8.23	5.48	4.19	3.83	21.70	29.75	26.82	19.95	8.95	2.38
NF:															
Riccardi et al. 1984:															
E	48.08	56.19	41.44	23.62	11.18	4.45	2.08								
O	34	42	46	37	7	13	8								
O/E	0.71	0.75	1.11	1.57	0.63	2.92	3.85								
P	30.73	46.75	43.65	31.50	18.88	9.51	4.98	12.48**							
Sergeyev 1975:															
E	9.72	18.08	12.32	8.80	4.64	1.68	0.74		1.80	15.38	17.79	10.69	7.18	3.18	
O	3	15	16	15	5	2	0		1	11	20	12	7	5	
O/E	0.31	0.83	1.30	1.70	1.08	1.19	0.0		0.56	0.72	1.13	1.12	0.98	1.57	
P	6.82	15.24	12.37	10.52	6.61	2.85	1.58		1.31	12.82	16.90	11.57	8.86	4.53	1.36
Total:															
E	57.80	74.28	53.76	32.42	15.83	6.13	2.82								
O	37	57	62	52	12	15	8								
O/E	0.64	0.77	1.15	1.60	0.76	2.45	2.84								
P	37.70	61.87	55.87	42.03	25.60	12.38	7.55	11.24*							
AP:															
Murdoch et al. 1970:															
E	22.08	28.80	23.60	16.55	8.96	3.99	2.05		12.52	33.71	28.57	18.19	10.49	3.53	
O	6	16	26	18	24	11	5		2	17	22	31	26	6	
O/E	0.27	0.56	1.10	1.09	2.68	2.76	2.44		0.16	0.50	0.77	1.70	2.48	1.70	
P	9.02	17.61	20.98	21.40	16.85	10.90	9.23	7.87	4.14	17.70	23.82	24.08	22.05	12.21	7.13

Table 4 continued on next page

TABLE 4 (Continued)

Syndrome, Source and Distribution	Paternal Age								Maternal Age						
	<24	25–29	30–34	35–39	40–44	45–49	>50	χ^2_5	<19	20–24	25–29	30–34	35–39	>40	χ^2_4
Stevenson 1957:															
E	6.76	12.60	12.60	8.10	3.86	1.43	0.64		2.12	7.36	14.35	13.85	6.53	1.75	
O	2	4	11	10	10	4	5		2	4	4	22	11	4	
O/E	0.30	0.32	0.87	1.23	2.59	2.80	7.81		0.94	0.54	0.28	1.59	1.67	2.29	
P	1.63	5.48	9.43	10.43	8.57	5.44	5.02	1.38	0.62	3.39	10.36	15.70	11.71	5.21	9.94*
Total:															
E	23.84	41.40	36.20	24.64	12.82	5.41	2.69		14.64	41.07	42.92	32.04	17.07	5.28	
O	8	20	37	28	34	15	10		4	21	26	53	37	10	
O/E	0.28	0.48	1.02	1.14	2.65	2.77	3.72		0.27	0.51	0.61	1.65	2.17	1.89	
P	10.26	23.18	30.18	31.97	25.32	16.27	14.14	6.94	4.72	20.83	34.27	40.26	33.75	17.18	9.45
Marfan:															
Murdoch et al. 1972:															
E	5.42	7.00	5.27	3.06	1.45	0.54	0.27		2.50	7.27	6.49	4.20	2.01	0.54	
O	0	3	10	4	3	0	3		1	3	7	8	3	1	
O/E	0.0	0.43	1.90	1.31	2.07	0.00	11.11		0.40	0.41	1.08	1.90	1.50	1.85	
P	1.66	3.74	4.73	4.60	3.70	2.28	2.33	10.36*	1.07	4.58	6.00	5.71	4.00	1.63	2.13
Pfeiffer:															
Present study:															
E	6.14	6.38	4.10	2.02	0.87	0.32	0.17		2.61	7.08	5.77	3.00	1.23	0.31	
O	2	2	6	3	4	2	1		0	5	3	8	4	0	
O/E	0.33	0.31	1.46	1.49	4.60	6.25	5.88		0.0	0.71	0.52	2.67	3.24	0.02	
P	1.90	3.71	4.18	3.63	2.76	1.76	2.06	2.83	0.83	3.88	5.44	4.85	3.44	1.56	5.94
Crouzon:															
Present study:															
E	6.75	7.02	4.51	2.22	0.96	0.35	0.18		2.87	7.78	6.35	3.30	1.36	0.35	
O	4	5	8	2	1	1	1		0	7	8	5	2	0	
O/E	0.59	0.71	1.77	0.90	1.04	2.86	5.56		0	0.90	1.26	1.52	1.47	0	
P	4.06	5.88	5.06	3.36	1.95	0.95	0.75	2.94	1.74	6.14	6.53	4.42	2.37	0.80	3.13

Note.—Syndrome acronyms are as in table 2.
* $P < .05$.
** $P < .025$.

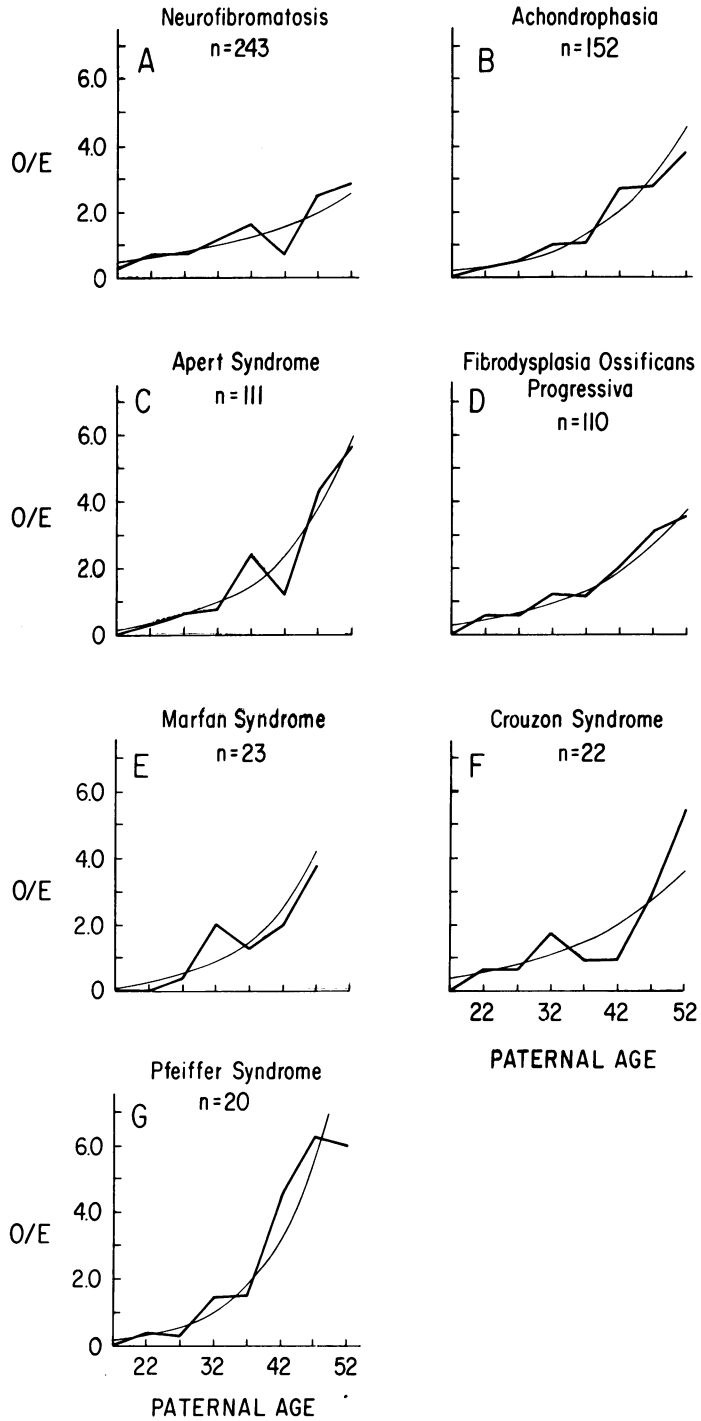

FIG. 1.—Relationship between O/E ratio and paternal age for seven syndromes

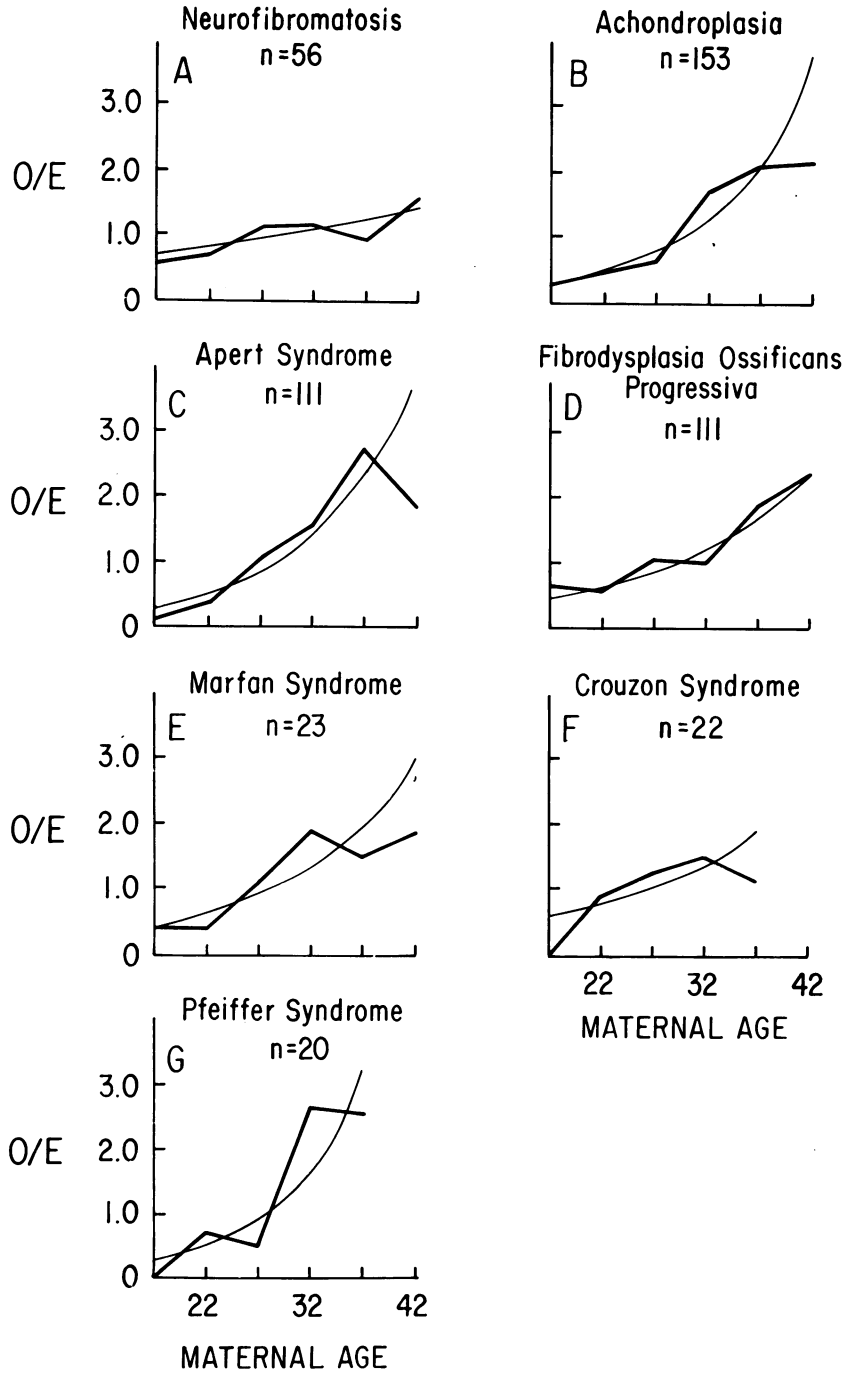

FIG. 2.—Relationship between O/E ratio and maternal age for seven syndromes

Exponential Model

Results of fitting an exponential model separately for fathers and mothers are given in table 5. The estimate of a and its SE are given for each study separately. For multiple samples of the same syndrome, combined estimates are also given, as well as a χ^2-test of heterogeneity among samples. In no case was there significant heterogeneity among studies of the same syndrome.

In figures 1 and 2 the best-fitting exponential curve is plotted along with the observed O/E ratio. Also, in table 4, the predicted (P) number of parents to fall in each 5-year age interval for the exponential model was calculated, and a χ^2 goodness-of-fit was performed. In general, the exponential model appears to give an adequate fit to most data. However, there are a couple of observations worth noting. First, for Apert syndrome, there appears to be an excess of observed fathers between ages 35 and 39 and a deficit of fathers between ages 40 and 44; in fact, the observed curve for O/E reaches an initial peak at age 37 and drops at age 42 before rising again at age 47 (fig. 1). This deviation from an exponential model is significant by the χ^2 goodness-of-fit test ($P < .025$). It is important to note that this pattern was observed in *all three studies* contributing to the Apert total (see table 4). In all three cases, the O/E ratio peaks at age 37, drops at age 42, and rises again at age 47.

Except that the overall rate of increase is lower than in Apert syndrome, an identical pattern is observed for neurofibromatosis fathers: O/E peaks at age 37, drops at age 42, and rises again at age 47. Again, this pattern leads to a rejection of an exponential model by χ^2 goodness-of-fit ($P <. 05$).

For the remaining five syndromes there is a similar pattern, except that it occurs 5 years earlier and in no case leads to rejection of an exponential model. For Marfan and Crouzon syndromes, there is an initial peak at age 32 and a drop at age 37 before another rise at age 47. For the remaining three syndromes, there is a plateau from age 32 to age 37 before another increase at age 47.

Among mothers, the only significant deviation between expected and observed ages is for achondroplasia, for which there is an observed deficiency of mothers >40 years of age. The same deficiency appears to exist for Apert and Marfan syndromes (although it is not significant). This observation is, in some sense, not surprising. If a major effect is due to an exponential model in fathers, then we would expect to see less than an exponential rate of increase in mothers, particularly at more advanced ages, when the correlation between fathers' and mothers' ages diminishes. An exponential curve in mothers would only result if the bivariate distribution of parental ages were truly normal.

Heterogeneity among Syndromes

Table 5 demonstrates quite a range of estimates of a, both in fathers (.025–.113) and mothers (.015–.125). We performed a χ^2-test of heterogeneity for the 16 syndromes listed in table 5. For both fathers and mothers, heterogeneity among syndromes was significant ($\chi^2_{16} = 45.91$, $P < .001$ for fathers; and $\chi^2_{16} = 42.37$, $P = <.001$ for mothers).

TABLE 5

RESULTS OF FITTING EXPONENTIAL MODEL

SYNDROME AND SOURCE	$\hat{a}$ (SE)	
	Paternal	Maternal
Apert:		
Present study	0.111(0.021)	0.138(0.029)
Blank 1960	0.094(0.020)	0.104(0.026)
Cohen 1975	0.089(0.016)	0.083(0.021)
Total	0.095(0.011)	0.101(0.014)
Heterogeneity χ^2 (df = 2)	0.71	2.36
FOP:		
Connor and Evans 1979	0.073(0.022)	0.084(0.028)
Rogers and Chase 1979	0.058(0.019)	0.052(0.024)
Tunte et al. 1967	0.097(0.026)	0.077(0.032)
Tunte et al. 1967	0.068(0.032)	0.040(0.040)
Total	0.071(0.012)	0.063(0.015)
Heterogeneity χ^2 (df = 3)	1.48	1.28
AP:		
Murdoch et al. 1970	0.075(0.011)	0.093(0.014)
Stevenson 1957	0.109(0.017)	0.090(0.025)
Total	0.084(0.009)	0.091(0.012)
Heterogeneity χ^2 (df = 1)	2.82	0.01
NF:		
Riccardi et al. 1984	0.047(0.009)	
Sergeyev 1975	0.040(0.017)	0.026(0.020)
Total	0.045(0.008)	
Heterogeneity χ^2 (df = 1)	0.13	
Crouzon:		
Present study	0.059(0.026)	0.053(0.034)
Jones et al. 1975	0.081(0.018)	0.074(0.023)
Total	0.073(0.015)	0.067(0.019)
Heterogeneity χ^2 (df = 1)	0.29	0.26
Marfan:		
Murdoch et al. 1972	0.103(0.023)	0.077(0.032)
Pfeiffer:		
Present study	0.113(0.024)	0.108(0.034)
BCN:		
Jones et al. 1975	0.104(0.031)	0.125(0.044)
Progeria:		
Jones et al. 1975	0.083(0.026)	0.097(0.033)
Waardenburg:		
Jones et al. 1975	0.078(0.024)	0.097(0.032)
CCD:		
Jones et al. 1975	0.071(0.020)	0.099(0.025)
AD:		
Jones et al. 1975	0.069(0.034)	0.042(0.045)
ODD:		
Jones et al. 1975	0.060(0.035)	0.042(0.045)
Sotos:		
Jones et al. 1975	0.052(0.017)	0.079(0.020)
T-C:		
Jones et al. 1975	0.045(0.012)	0.040(0.015)
ME:		
Jones et al. 1975	0.035(0.033)	0.019(0.042)
BR:		
Pellie et al. 1973	0.025(0.011)	0.015(0.013)
Total heterogeneity among syndromes χ^2_{16}	45.91*	42.37*

NOTE.—Syndrome acronyms are as in table 2.

* $P < .001$.

TABLE 6

RESULTS OF ANALYSIS OF HETEROGENEITY AMONG SYNDROMES

PARAMETER	PATERNAL		MATERNAL	
	1-*A* Model	2-*A* Model	1-*A* Model	2-*A* Model
p_1	1.0	0.321(0.153)	1.0	0.285(0.154)
A_1	0.0644(0.0036)	0.0418(0.0060)	0.0654(0.0049)	0.0297(0.0108)
A_2	...	0.0827(0.0052)	...	0.0841(0.0069)
$-2 \ln L$	90.27	110.94	83.90	100.68

To determine the minimum number of a's necessary to account for the observed variation among syndromes, we applied the mixture analysis described above in Methods. The results are given in table 6. For fathers, the model assuming two exponential parameters was significantly superior to the model assuming one parameter ($\chi^2_2 = 20.67$, $P < .001$). However, a model allowing for three exponential parameters converged to the two-parameter model. Hence, the variation among syndromes could be explained by two parameters (with values .042 and .083, respectively). Among mothers, the results were similar. A two-parameter model was significantly superior to a single-parameter model ($\chi^2_2 = 16.78$, $P = <.001$); the three-parameter model offered no improvement over the two-parameter model.

The majority of syndromes—68% in males and 71% in females—are estimated to belong to the high-parameter group. This difference between males and females is not significant. Interestingly, the estimate of the larger exponential parameter is nearly double the value of the smaller exponential parameter in males. It is also interesting that the larger A value is greater in mothers than in fathers, whereas the smaller A value is greater in fathers than in mothers.

Mutation-Heterogeneity Analysis

Using maximum likelihood, we applied the mutation-heterogeneity model (i.e., $\pi(i) = d + ce^{ai}$), to each of the seven syndromes listed in table 4. In every case, the model converged to a pure exponential model (i.e., $d = 0$). Hence, on the basis of this analysis, there is little evidence for a sizable subset of parental age–unrelated cases for any of the syndromes. This result is not surprising, considering the curves in figures 1 and 2. If there were a subset of age-independent cases, then the observed O/E curves would be relatively flatter at younger ages and closer to exponential at older ages—a pattern seen, for example, for some trisomies vis-à-vis maternal age (Risch et al. 1986). Our inability to detect age-unrelated cases in the present study cannot be attributed to small sample size, at least in the cases of Apert syndrome and achondroplasia; for example, for both of these syndromes, any model specifying $\geq 20\%$ age-independent cases would be rejected by a likelihood-ratio criterion.

Paternal versus Maternal Age

The results of the analysis of the paternal effect (p = 1), maternal effect (p = 0), and mixed-effect (p = .5) models are given in table 7. The estimate of a and

TABLE 7

EXPONENTIAL MODEL PREDICTIONS FOR MATERNAL AGES GIVEN OBSERVED PATERNAL AGES

SYNDROME: SOURCE	MODEL									
	Paternal Effect (p = 1.0)			Mixed-Effect (p = .5)			Maternal Effect (p = 0)			
	a	μ_M^e	Z	a	μ_M^e	Z	a	μ_M^e	Z	$\hat{p}$
Apert: present study	.112	29.6	+1.55	.129	30.4	+0.66	.197	33.6	−2.88	0.3
Pfeiffer: present study	.112	29.6	0.00	.129	30.4	−0.78	.197	33.6	−3.88	1.0
BCN: Jones et al. 1975	.104	30.4	+0.98	.120	31.2	+0.38	.184	34.3	−1.96	0.3
Marfan: Murdoch et al. 1972	.103	30.3	−1.05	.116	31.1	−1.89	.176	33.9	−4.82	1.0
Apert: Blank 1960	.094	31.0	+1.11	.117	31.8	0.00	.175	34.4	−3.61	0.5
Apert: Cohen 1975	.089	29.9	−0.61	.108	30.7	−1.82	.153	33.0	−5.32	1.0
Progeria: Jones et al. 1975	.083	28.9	+0.76	.096	29.5	+0.19	.130	31.4	−1.63	0.4
Crouzon: Jones et al. 1975	.081	28.7	−0.14	.093	29.4	−1.12	.126	31.2	−3.63	1.0
AP: Murdoch et al. 1970	.075	29.8	+1.72	.091	30.6	0.00	.131	32.7	−4.52	0.5
Waardenburg: Jones et al. 1975	.077	29.3	+1.28	.091	30.0	+0.53	.130	31.9	−1.49	0.3
FOP: Connor and Evans 1979	.073	30.0	+0.54	.085	30.6	−0.27	.120	32.2	−2.43	0.7
CCD: Jones et al. 1975	.071	28.3	+1.96	.082	28.9	+1.18	.109	30.3	−0.65	0.1
AD: Jones et al. 1975	.069	28.2	−0.08	.081	28.8	−0.54	.107	30.2	−1.61	1.0
NF: Riccardi et al. 1984	.064	28.2	−2.51	.075	28.7	−4.07	.101	29.9	−7.83	1.0
FOP: Rogers and Chase 1979	.058	28.4	+0.15	.070	28.9	−0.60	.093	30.1	−2.41	0.8
ODD: Jones et al. 1975	.060	27.8	+0.24	.070	28.3	−0.16	.092	29.4	−1.02	0.7
Crouzon: present study	.059	27.5	−0.48	.070	28.0	−1.03	.095	29.2	−2.53	1.0
Sotos: Jones et al. 1975	.052	27.4	+2.38	.061	27.9	+1.53	.078	28.8	0.00	0.0
T-C: Jones et al. 1975	.045	27.1	−0.24	.053	27.5	−1.21	.067	28.3	−3.15	1.0
NF: Sergeyev 1975	.040	29.8	+0.18	.047	30.2	−0.53	.056	30.8	−1.59	0.9
ME: Jones et al. 1975	.035	26.7	−0.56	.042	27.1	−0.93	.053	27.6	−1.40	1.0
BR: Pellie et al. 1973	.025	28.4	−0.62	.031	28.7	−1.54	.041	29.1	−2.77	1.0
Total			1.40(1.98[a])			−2.56(−1.73[a])			−13.04(−11.63[a])	

NOTE.—Syndrome acronyms are as in table 2.

[a] Excluding Riccardi et al. (1984).

the expected mean maternal age (given the observed paternal age) are listed, along with corresponding Z-scores. We have performed all statistical analyses both including and excluding the study of Riccardi et al. (1984). In that study, the authors calculated a mean paternal age of 32.8 and a mean maternal age of 27.4. They also presented the distribution, in 5-year intervals, of paternal age. From that distribution, we calculated a mean paternal age of 32.3, 0.5 years less than that reported by Riccardi et al. (1984). No age distribution was presented for mothers. Because of this uncertainty—in addition to the fact that that study has the largest number of cases (thereby tending to dominate our analyses)—we decided to perform all analyses with and without that study. To be conservative, we used the mean paternal age reported by the authors, 32.8.

Overall, the paternal effect model appears to underestimate observed mean maternal ages. Considering all syndromes, the total Z-score,

$$\sum_{i=1}^{23} Z_i/\sqrt{23} \ ,$$

is 1.40, which corresponds to $P = .08$. Excluding the study of Riccardi et al. (1984), total $Z = 1.98$, for which $P = .02$. The maternal effect model clearly overpredicts maternal ages given the observed paternal ages, and this model can be rejected overall ($Z = -13.04$, $P < .001$). The mixed-effect model, with $p = .5$, also tends to overestimate mean maternal ages ($Z = -2.56$, $P = .005$; $Z = -1.73$, $P = .04$ without the Riccardi et al. [1984] study). Considering all syndromes, we also calculated a median value for p from the last column in table 7; this turns out to be .85.

We also divided the syndromes into two groups on the basis of their estimated a values from the paternal effect model. We denote as group 1 those syndromes with the higher A value, and as group 2 those with the lower A value. Using the observed A value and its SE for a given syndrome, we classified a syndrome in group 1 if its posterior probability of being in that group was $>.5$; otherwise, it was placed in group 2. Using this procedure, we placed the following syndromes in group 1: acrodysostosis, achondroplasia, Apert, basal cell nevus, cleidocranial dysostosis, Crouzon, fibrodysplasia ossificans progressiva, Marfan, oculo-dento-digital, Pfeiffer, Progeria, and Waardenburg. The group 2 syndromes were as follows: multiple exostoses, neurofibromatosis, retinoblastoma, Sotos, and Treacher-Collins. The total Z-score for the paternal effect model for group 1 syndromes was 1.98 ($P = .024$), whereas for group 2 syndromes $Z = -0.56$. Hence, the paternal effect model is less compatible with group 1 syndromes than with group 2 syndromes. Within the mixed-effect model ($p = .5$), $Z = -1.31$ ($P = .10$) for group 1, whereas $Z = -2.72$ ($P = .003$) for group 2. The median estimated p value for the group 1 syndromes is 0.7, whereas the median value for group 2 syndromes is 1.0. These results suggest the possibility that syndromes with smaller a values may result from mutations primarily of paternal origin, whereas those with larger a values may contain a significant proportion that are of maternal origin.

TABLE 8

EXPECTED PARENTAL AGE DIFFERENCE FOR THE MIXED-EFFECT MODEL FROM THE 1955 U.S. CENSUS

PATERNAL AGE	p = 1.0		p = .5		p = 0	
	$\mu_F - \mu_M$	a	$\mu_F - \mu_M$	a	$\mu_F - \mu_M$	a
30	3.5	.002	3.5	.002	3.5	.002
31	3.8	.020	3.6	.024	3.3	.031
32	4.2	.037	3.9	.044	3.2	.059
33	4.6	.053	4.2	.062	3.1	.084
34	5.1	.067	4.5	.079	3.0	.110
35	5.5	.080	4.9	.094	2.9	.135
36	6.0	.093	5.3	.107	2.8	.160

We note here that although it is easy to distinguish between the paternal effect model and the maternal effect model, it is considerably more difficult to distinguish between a paternal effect model (p = 1) and a mixed-effect model (p = .5). The reason for this can be seen in table 8. For a given observed paternal age, the expected difference between mean paternal and maternal ages is quite different for a paternal effect model versus a maternal effect model, especially at high parental ages. However, the difference between a paternal effect model and the mixed-effect model is quite small, even at high ages; for example, for an observed mean paternal age of 36, the expected difference between the predicted differences for the two models is only 0.7 years.

DISCUSSION

What do these results tell us about the process of spontaneous mutation in humans and its relationship with parental age? Two conclusions are clear: (1) Among dominant mutations, there is heterogeneity in the relationship between mutation incidence and parental age. The simplest model compatible with the data specifies two distinct groups of mutation with different rates of increase. (2) The group with higher rate of increase is not compatible with a linear model relating incidence to age; the group with lower rate of increase may be compatible with a linear model; however, it is also consistent with an exponential model. In this analysis, the larger percentage of syndromes belongs to the high-rate-of-increase group. These results confirm statistically an earlier (Vogel 1964) impression of heterogeneity.

The linear model for males assumes that the rate of spermatogenesis is constant with age. This assumption may or may not be true. It has been suggested that the rate of spermatogenesis may actually decline with increasing age; on the other hand, the possibility also exists that the decrease represents a diminution in the number of dividing stem cells and not in the rate of division among surviving ones (Vogel and Rathenberg 1975).

In the high-rate group, the evidence for a strict paternal age effect and no maternal age effect is not compelling. The data are equally (or more) consistent with an exponential model in which both sexes show an equal age effect. In the mixed-effect model analysis, the median estimated value of p (prior probability

of mutation in males) was .7. In the group with a low rate of increase, the median p value was 1.0. This group appears to be compatible with mutation occurring primarily in males. In the heterogeneity analysis, we assumed that syndromes belonged to one of two groups, each with a unique a value. In other words, we assumed that the prior distribution of a values was discrete, taking on a finite number of possible values. We also analyzed the data assuming a normal prior distribution (i.e., continuous) for a values. In this case, there are two parameters to estimate, the prior mean and variance. As expected, the prior variance was estimated as significantly greater than zero, owing to heterogeneity among syndromes (for both males and females). The log likelihood for this model was 53.71 in males and 49.24 in females. These values are less than those for the 2-A models; however, a formal statistical comparison is not possible. Hence, while two distinct groups of mutations with corresponding A values is a reasonable conjecture, the possibility of a continuity of A values among syndromes cannot be ruled out.

In performing the analyses on paternal versus maternal age, we assumed an exponential model. Assuming some type of model was necessary for those syndromes for which we only had mean parental ages. However, for those studies that gave the list of parental ages, it is possible to calculate exactly the expected maternal ages given the observed paternal ages from the control bivariate age distribution. As a check, we compared, when possible, the expected mean maternal ages derived directly from the paternal ages with those obtained by assuming the exponential model. In general, the differences were small, with an average difference <0.1 years. Hence, the results of this analysis are unlikely to have been much influenced by the exponential assumption.

For those syndromes for which we had distributional data on parental ages, there was no evidence for admixture of exponentially age-dependent and age-independent cases. If one were to postulate that both father and mother contribute substantially to the mutation load for these syndromes, then the maternal cases must also increase in frequency with age, as do the paternal cases. For the group of syndromes with a high rate of increase with age, the model based purely on paternal ages is rejected. Therefore, the conclusion most compatible with this group of syndromes is that fathers and mothers both contribute mutations (on average 70% and 30%, respectively) and that the incidence increases with age in both sexes. Although we assumed the same rate of increase with age in males and females in our modeling, the rates may in fact be different. Under the circumstances, however, such differences would be impossible to detect. Also, these results do not rule out the possibility of etiological heterogeneity of mutation for some of the syndromes not examined. For example, molecular heterogeneity of the mutational defect of a number of syndromes (e.g., retinoblastoma, Duchenne muscular dystrophy, and thalassemia) has been identified.

There appears to be no relationship between rate of increase of mutation incidence with parental age and absolute mutation rate for a given syndrome. For example, neurofibromatosis has a high overall mutation rate (10^{-4}) but a low rate of increase with age. Treacher-Collins syndrome has a low overall

mutation rate and a low rate of increase. Achondroplasia has a higher overall mutation rate and a high rate of increase, whereas Apert and Marfan syndromes have high rates of increase but lower overall mutation rates (Vogel and Rathenberg 1975). Hence, absolute mutation rate does not seem to offer additional insights into the mechanism of mutation and relationship to parental age. There also appears to be no clear syndrome-type pattern distinguishing the high- and low-rate groups.

The particular patterns observed for Apert syndrome and neurofibromatosis—namely, the early peak at age 37 and the drop at age 42—are difficult to explain, especially according to a copy-error model or any other model that assumes accumulation of mutation in stem cells with age during spermatogenesis. According to any such model, the curve would have to be monotonically nondecreasing. Although for both syndromes a simple continuous model was rejected by a χ^2 goodness-of-fit test, the observed pattern might be artifactual. Arguing against this, however, is the identical pattern observed in all three studies of Apert syndrome. The other syndromes did not have the exact same pattern, but they did tend to show, relative to what would be expected, an excess of cases at age 32 and a deficit at age 37, although differences were never significant. One might conjecture (1) that stem cells divide at different rates, with those undergoing more divisions dying earlier, or (2) that a fresh collection of undivided stem cells is recruited in early middle age. However, if this were the case, we would expect the different types of mutation to show a similar pattern. Also, differences cannot be explained by division of syndromes into high-rate-of-increase and low-rate-of-increase groups; Apert syndrome shows a high rate of increase with paternal age, whereas neurofibromatosis shows a low rate of increase.

It may well be the case that all dominant syndromes show at least some parental age effect; for example, it was originally thought that sporadic cases of bilateral retinoblastoma and osteogenesis imperfecta did not increase with parental age. However, the most recent and most extensive reports on these two syndromes (Pellie et al. 1973; Carothers et al. 1986) do indicate a minor but significant parental age effect.

Although in our analysis we employed an exponential model, which generally gave an acceptable fit to the data, this does not imply that the exponential model is the only possible acceptable model. For example, a power model—$[\pi(t) = t^{\alpha}]$—was fit to those studies that reported complete data. A median value of $\alpha = 2.0$ was obtained for the high-rate-of-increase group. Hence, at this stage, the heterogeneity in the data may also be compatible with a one-step or two-step model of mutagenesis.

In conclusion, our analyses confirm skepticism regarding a simple copy-error model as being the primary source of spontaneous mutation in humans. The majority of syndromes examined showed a significantly greater than linear increase with parental age. Also, the evidence appears to be against increased age in fathers as the only source of the parental age effect, and the patterns of some curves are inconsistent with the monotone increase required by a mutation-accumulation model. A possible model that could explain these results is

one that specifies an increased probability of mutation with time spent by a spermatozoon or ovum in a haploid state, a period of time that may also increase with age of the parent. A firm answer to the question of parental age and new mutation awaits identification of the molecular defect underlying some of these syndromes; we will then be in a position to determine in which parent the mutation occurred and at what age it did so.

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